THE STRUCTURE OF MEIOTIC CHROMOSOMES IN THE GRASSHOPPER AND ITS BEARING ON THE NATURE OF "CHROMOMERES" AND "LAMP-BRUSH CHROMOSOMES"

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The nature of the gene is one of the fundamental problems in modern biology. Since the genes are located in the chromosomes, the structure, chemistry, and metabolism of the chromosomes are of special significance for the understanding of the gene and gene action. The prevalent interpretation of chromosome structure has developed as a kind of compromise between two originally opposed views, the "chromomere hypothesis" of Balbiani, Pfitzner, and Strasburger and the "chromonema hypothesis" of Baranetzky, Bonnevie, and Vejdovsky.[2] According to the "chromomere hypothesis," the chromosome consists of a series of small beads or discs strung together. During prophase they approach each other, fuse into larger complexes, and finally disappear in the thick rod-shaped metaphase chromosomes. For the "chromonema hypothesis" on the other hand, the fundamental unit of the chromosome is a coiled thread, tightly wound in a helix at metaphase and more or less uncoiled during interphase. Both chromomeres and spirals were discovered about the same time (Balibiani, 1876; Pfitzner, 1882; Baranetzky, 1880). Yet more and more structures first described as "chromomeres" have turned out to be coils and today the "chromomere" is in full retreat into the submicroscopic level. Strasburger's "chromomeres" in Tradescantia pollen mother cells had been clearly shown to be spirals by Baranetzky (1880); Pfitzner's "granules" in somatic prophases of the salamander were resolved into coils by Schneider (1910) and by Lee (1921), who concluded that all "chromomeres" are in reality turns in the helix. The modern view which is accepted by most cytologists today and is based mainly on Heitz (1935), holds that the true "chromomeres" (Belling's ultimate chromomeres) can only be seen in the prophase of meiosis (leptotene) and in the curious giant chromosomes of dipteran larvae, where the chromonemata are assumed to be completely uncoiled. According to this view (Reuter, 1930; Heitz, 1935; Darlington, 1937; White, 1937; Geitler, 1938; Koltzoff, 1938; Kuwada, 1939; Nebel, 1939; Huskins, 1941, 1942; Straub, 1943) the chromonema consists of chromomeres of different but constant size, rich in nucleic acid, connected by protein fibrils. The chromomeres bear the genes, they reproduce as specific units and they synapse in meiotic prophase. They are the visible expression of the linear arrangement of the genes.

[1] Part of the work for this paper was done in the Department of Biology, Johns Hopkins University.

[2] The "vacuolization hypothesis" of Grégoire and his school, denying both chromomeres and chromonemata, has been thoroughly disproved by the work of the last twenty years and need not be discussed here.

Yet even in leptotene chromosomes the "chromomeres" were found to be coils by several authors. They were first described as such in Tradescantia by Kaufmann (1931), who nevertheless accepted the "chromomere" interpretation for other plants and animals (Kaufmann, 1936). Koshy (1934, 1937) found the leptotene chromosome to be coiled in Allium and Aloe, Naithani (1937) in Hyacinthus. Smith (1932) suggested that the beadlike appearance of the leptotene in Galtonia might be due to twists in the chromonema and Hoare (1934) noted that the zygotene threads give the impression of two tightly coiled chromonemata. Kuwada (1939) pointed out that sharp turns in the coils might easily be mistaken for "chromomeres." In Tradescantia, Swanson (1943) found no "chromomeres" which could not be resolved into coils, and he suggested that a chromomere pattern such as that in maize might be due to differential spiralization.

Yet most recent discussions on the gene and chromosome structure cling tenaciously to the belief that "chromomeres" are real (e.g., Schultz, 1944). The main evidence usually presented, besides the salivary chromosomes of dipteran larvae, is the observations of Wenrich (1916), Lewis and Robertson (1916), and Chambers (1924) on the large chromosomes in grasshopper spermatocytes. To re-examine this evidence is the purpose of the present investigation.

Material and Methods

Spermatocytes of *Chorthippus curtipennis, Chorthophaga viridifasciata, Dissosteira carolina, Melanoplus femur-rubrum, Arphia* sp., *Hippiscus* sp., and *Orphulella* sp. were studied in sections (fixation: B 15 and Sanfelice, stain: Feulgen), and aceto-orcein smears. For the detailed study of leptotene chromosomes sections stained with Feulgen were found to be more reliable than smears. To uncoil chromosomes, testes were submersed for one-two hours in $2 \cdot 10^{-3}$ M KCN in Bělař solution (Bělař, 1929) before smearing (Oura, 1936). The optics used consisted of a Zeiss aplantic condenser N.A. 1.4, Zeiss 2 mm. objective N.A. 1.4 and 15× ocular. The photographs (except Figure 12) were taken with the same optics and a Bausch and Lomb photomicrographic camera type H. The stereoscopic photographs were made by shifting the substage diaphragm maximally to the left and right respectively for the two exposures.[3]

The Structure of Leptotene Chromosomes

On casual examination the slender, irregularly twisted chromosomes at leptotene have a beaded appearance as has been so often described in the literature (for a review see Reuter, 1930). A detailed study with the best optics and a delicate use of the fine adjustment screw of the microscope, however, resolves the beads or "chromomeres" into turns of a narrowly pitched coil[4] (Figures 1, 6a, and 13). With Feulgen the chromosome stains evenly throughout its length and there are no Feulgen-negative "interchromomeric fibrils." This uniform nature of the

[3] I wish to thank Mr. John Spurbeck, Dept. of Biology, Johns Hopkins University, for help with the photomicrographs.

[4] Mr. L. Vanderlyn, Dept. of Zoology, University of Pennsylvania, informs me that he has come independently to the conclusion that the "chromomeres" are in reality gyres in the chromonemata. In a forthcoming paper he will trace the origin of these from the unpacking coils of the preleptotene in *Podisma alpina.*

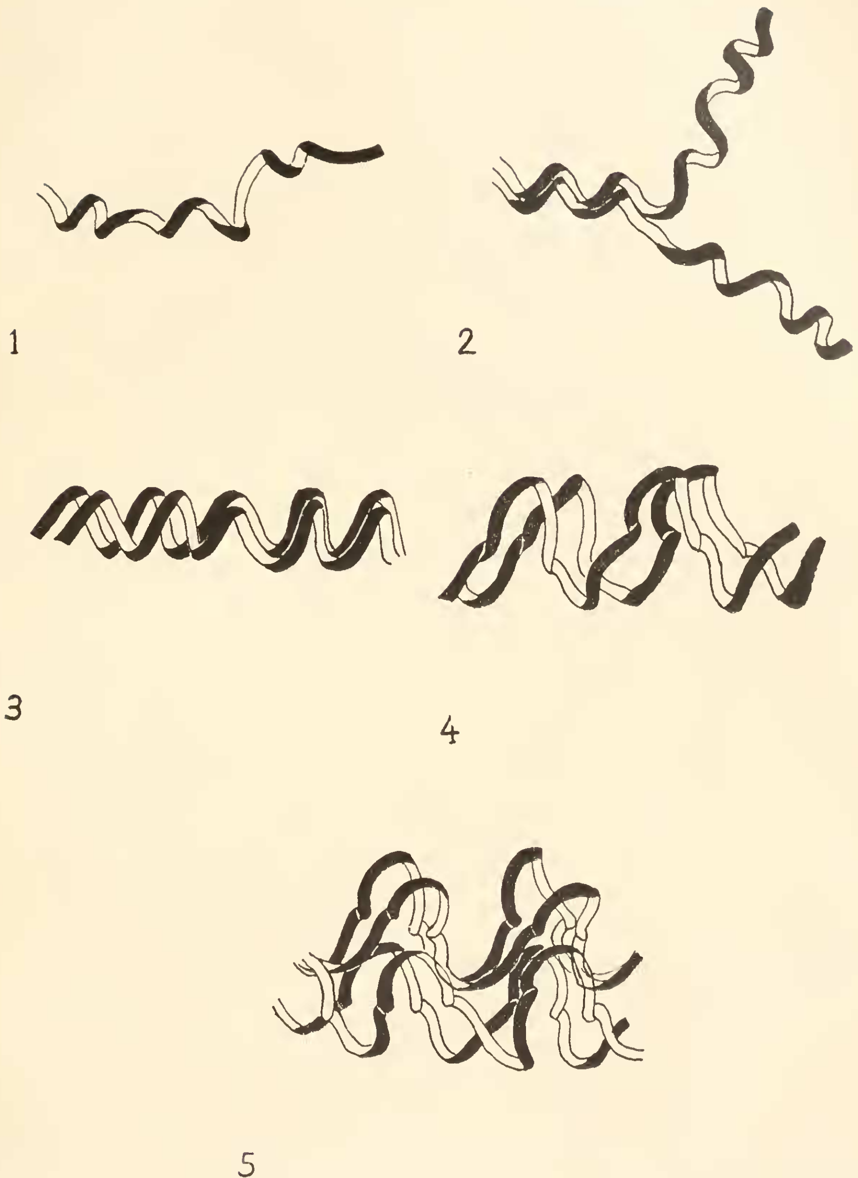
leptotene chromosomes can best be seen in well fixed sections. A chromosome, followed with the fine adjustment as it winds itself through the nucleus, is seen to be a thread of uniform thickness thrown into a tight, irregular helix. The narrow turns of this coil where the chromosome overlaps itself, appear as "chromo meres." The gyres can vary in width and may be unevenly spaced (see Figure 13). This can give the impression of different sized chromomeres. The width of the thread and the tightness of the helix are characteristic for each species of grasshopper studied. In aceto-orcein smears, when the chromosome has been under shear or pressure, an apparent chromomeric structure is more pronounced. This is due to the wax-like consistency of the chromosome which causes its gyres to fuse or be pulled out and otherwise distorted. Chromosomes, in which the coils can be clearly seen, can easily be transformed into the classical string of beads simply by exerting pressure on the coverslip and smearing them out. It is interesting to note in this connection that Belling (1931) emphasized that chromomeres are not clear in sections and that one has to use smears to make them visible.

When does that tight irregular coil of the leptotene chromosome originate? Is there any stage when the chromonemata are completely stretched out and without any signs of coiling? In all the grasshoppers studied no chromosome was found that did not show some degree of coiling. Furthermore, the characteristic coil of the leptotene chromosome is already present in the interphase and unravelling stage of preleptotene. We must assume that the leptotene spiral originates in the interphase or telophase of the preceding division. This origin of a prophase helix in the preceding telophase has been demonstrated by Sparrow (1942) in the microspore division in Tradescantia. The chromosome of the unravelling stage is thus doubly coiled (Figure 7). It shows the wide gyres of the previous metaphase relaxing into the relic coils of leptotene and the small tight helix which is destined to enlarge during pachytene and become the major coil of the first meiotic metaphase chromosome. This structure of the preleptotene chromosome was indicated clearly in McClung's figures for *Mecostethus lineatus* (esp. Figure 43, McClung, 1927). The heteropycnotic X chromosome in the prophase of grasshopper spermatocytes, which does not unwind in preleptotene and is thus comparable to the preleptotene autosomes in structure, similarly discloses a small tight helix and a wide irregular coil as Coleman (1943) has demonstrated.

Since the preleptotene chromosome consists of at least two chromonemata the leptotene chromosome also must be double (Robertson, 1931). The split between the chromatids can sometimes be discerned, especially in the turns of the coil, but usually the sister strands are closely appressed. They seem to form a plectonemic spiral, though this could not be determined with certainty.

The Structure of Zygotene Chromosomes

The pairing of homologous chromosomes at zygotene thus takes place between two coiled structures. The gyres of the two chromosomes fit into each other and become more or less closely appressed (Figures 2 and 6b). The bivalent now forms a paranemic coil. Just as the gyres in leptotene were mistaken for "chromomeres," so the gyres of the parallel coil in the bivalent were thought to be paired "chromomeres."



FIGURES 1–5. Diagrammatic representation of chromosome structure during meiotic prophase of the grasshopper.

FIGURE 1. Leptotene.

FIGURE 2. Zygotene.

FIGURE 3. Pachytene. The homologues can be either slightly separated or closely appressed.

FIGURE 4. Later pachytene. Appearance of the minor coil.

FIGURE 5. Diplotene. The chromonemata have separated laterally. This represents in essence also the structure of "lamp-brush chromosomes."

The Structure of Pachytene Chromosomes

During pachytene the helices of the paired chromosomes increase in width and the number of gyres decreases. This process is identical to that described by Swanson (1942a) for Tradescantia (despiralization cycle). If the chromosomes are closely appressed only one helix is visible. When the coils separate slightly a reticular or vacuolated appearance is produced, though often two parallel helices can be clearly discerned (Figures 3 and 8). In late pachytene an irregular waviness appears on the gyres of the pachytene coil; this sometimes looks like a very fine spiral of narrow pitch. It most likely corresponds to the minor spiral described in plant chromosomes (Figures 4 and 9).

The Structure of the Chromosomes During Diplotene and Diakinesis

In this stage the chromosomes are most difficult to analyze. They are usually described in the literature as diffuse, having fuzzy or woolly fringes (see for instance Nebel and Ruttle, 1937). The better the general fixation seems to be, the less distinct or sharp the chromosomes appear. However, after submersing the cells for one to two hours in $2 \cdot 10^{-3}$ M KCN in Bělař solution and staining in aceto-orcein, the structure of the diakinesis chromosome and the reason for its woolly appearance becomes quite clear. The lateral separation of the chromonemata which had already begun in pachytene has progressed much further, so that their gyres now overlap only within a narrow central region. This region appears as a beaded darker core of the chromosome. The gyres of the major coil of the chromonemata form loops projecting beyond this central core (Figures 5 and 14). It is these loops of the individual chromonemata which give the chromosome its hairy appearance. If the separation of the coiled threads is great the chromosome looks like a dark, beaded rod with loops or hairs at regular intervals (Figure 14a). When the lateral shifting is less the chromosome gives the impression of a double beaded rod, the loops or hairs now of course being shorter (Figure 14b). These appearances can easily be explained on a model of four simultaneously coiled wires. Sometimes one or more irregular turns of the minor coil can be seen on the loops.

In this stage there is further evidence against the reality of "chromomeres." If the apparent thickenings in the leptotene chromosome were constant units of definite size, they should be visible also in the loops of the diplotene chromatids.

Plate I

Figure 6. Chorthophaga, zygotene. Pretreated with ammonia vapor. Aceto-orcein smear. Note the coil of the univalent at *a* and the paranemic helix of the bivalent at *b*.

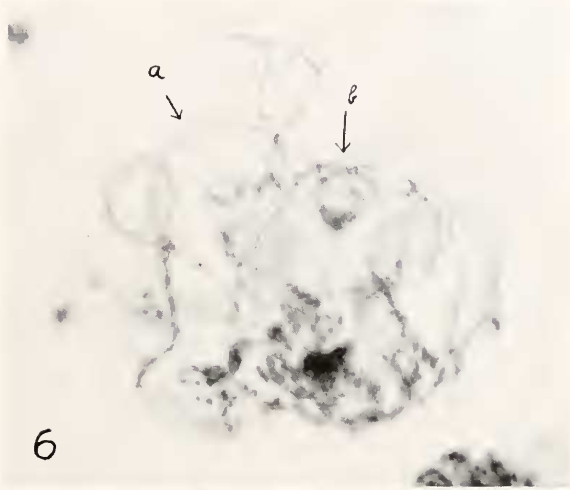
Figure 7. Chorthophaga, preleptotene. Aceto-orcein smear. Irregular "major coil" in the process of unravelling. The narrowly pitched helix ("minor coil") corresponds to the leptotene spiral (arrows).

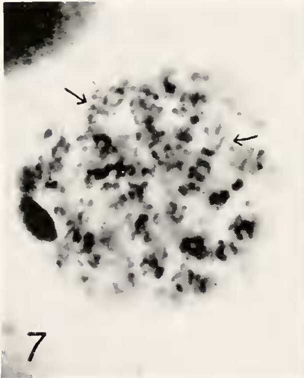
Figure 8. Chorthippus, early pachytene. Section. Fixed with Sanfelice and stained with Feulgen.

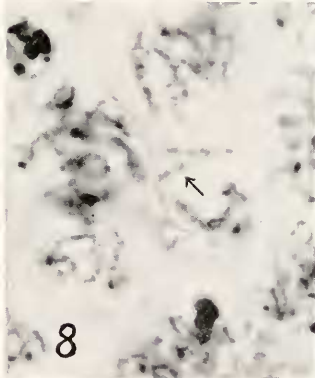
Figure 9. Hippiscus, late pachytene. Section. Fixed with Sanfelice and stained with Feulgen.

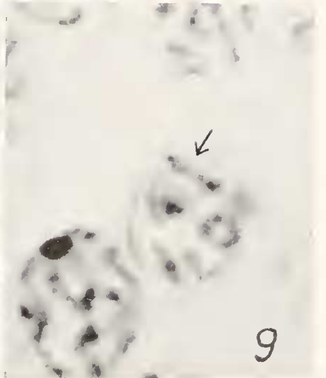
Figures 10 and 11. Orphulella, pachytene. Pretreated for 2 hours in KCN. Aceto-orcein smear. The heterochromatic knobs have been resolved into coils (arrows).

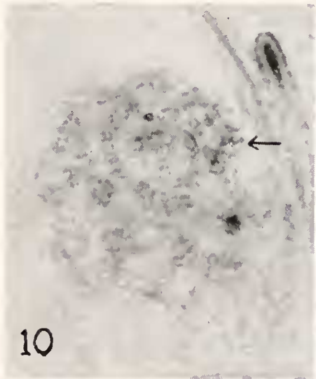
Figure 12. Fragment of a "lamp-brush chromosome" from a frog oöcyte. Aceto-orcein smear. Note the loops of the major coil and the minor coil (arrows). Zeiss 3 mm. objective, 15 × ocular.

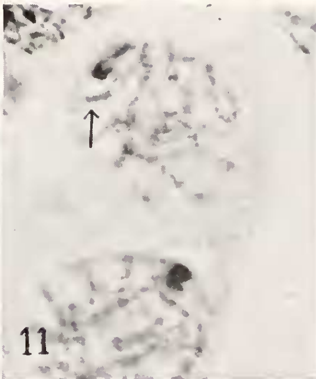












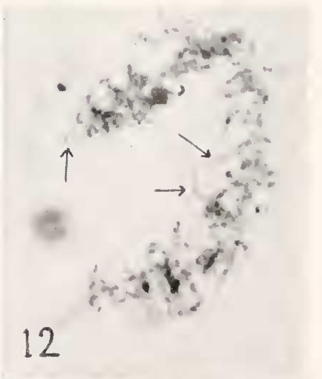


PLATE I

These chromatids, however, never show any beaded structure. The despiralization already noted in pachytene has continued and has resulted in an increase in width and decrease in the number of gyres with a consequent shortening and thickening of the chromosome.

The Structure of Metaphase Chromosomes

At the end of diakinesis the gyres of the chromatids become more closely spaced along the chromosome axis, leading to a further shortening of the chromosome and a fusion of the "chromatic coating" (Ris, 1942) of the individual chromatids, so that a uniformly staining body results. The chromatids retain their lateral separation, causing what is sometimes observed as a reticulate or vacuolated appearance of the metaphase chromosomes.

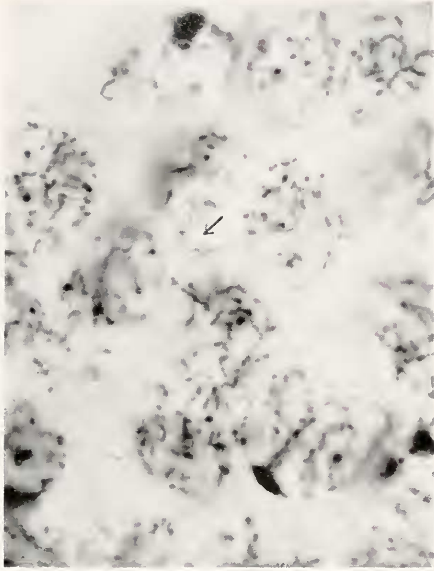
The Nature of the Heteropycnotic Regions in Orphulella

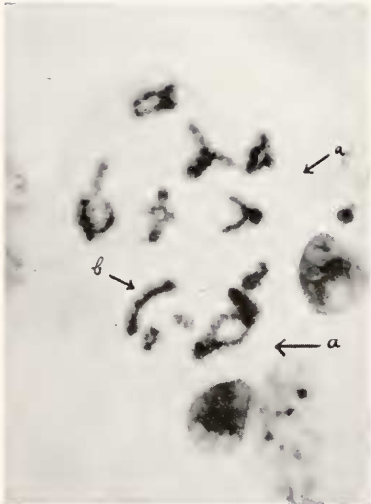
During meiotic prophase the chromosomes of Orphulella carry small, knob-like, darkly staining bodies, especially at their ends. These structures resemble the large "chromomeres" described by Wenrich (1916) in Phrynotettix. Treatment with KCN for 3 hours causes a loosening of the chromosome helix and shows that these knobs are tightly coiled regions of the chromosome (Figures 10 and 11). It is evident that the different appearance of such heteropycnotic regions in meiotic chromosomes is mainly due to differential coiling of the chromonemata as has been shown for the X chromosome by Coleman (1943). Similarly Wilson and Boothroyd (1944) have demonstrated that heterochromatic differentiations after cold treatment are the result of differential coiling.

Discussion

Chromomeres

The synthesis of cytology and genetics in the chromosome theory of inheritance has had a stimulating effect on the investigation of chromosomes. Yet the knowledge of the intimate structure of the chromosome has been retarded rather than furthered by the influence of genetics. The constant desire to find visual expression of the linear order of genes has led to the perpetuation of misinterpretations of the microscopic image. Indeed cytogenetics has established beyond doubt the longitudinal differentiation of chromosomes, but it is not justifiable to conclude that the units of this differentiation are microscopically visible particles. Thus observations which did not agree with the "chromomere" hypothesis tended to be ignored. The extensive literature on the subject (see Reuter, 1930) shows the widespread acceptance as well as the great versatility of the chromomere concept. Almost any expression of unevenness along the chromosome was at one time or other called "chromomere." The first pictures of "chromomeres" were published by Balbiani (1876) and Pfitzner (1882). Both described prophase and metaphase chromosomes in somatic cells. Today there can be no doubt that they saw the gyres of the somatic helix (Schneider, 1910; Lee, 1921; Creighton, 1938). Strasburger (1882) and Farmer and Shove (1905) described disc-like "chromomeres" in meiotic metaphase chromosomes of Tradescantia. We know now that they mistook the gyres of the major coil for discs. Quite often chromocenters in





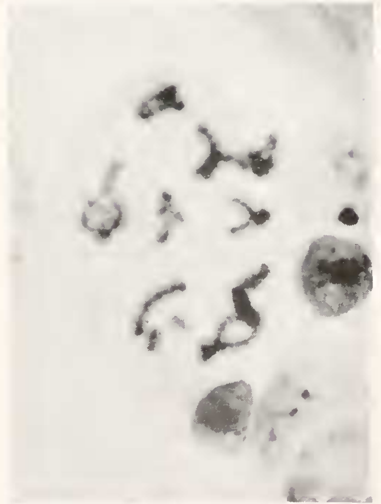


PLATE II

FIGURE 13. Stereophotomicrograph, Chorthippus leptotene. Section. Fixed with Sanfelice and stained with Feulgen and Iron hematoxylin. Note the coiled leptotene chromosomes (arrow).

FIGURE 14. Stereophotomicrograph, Hippiscus diakinesis. Pretreated with KCN. Aceto-orcein smear. Note the loops of the major coil which give the chromosomes at this stage the fuzzy appearance.

interphase nuclei and heteropycnotic regions on the chromosome, such as found in the X chromosome of *Notonecta indica* (Browne, 1916), were called "chromomeres" (cf. Heitz, 1929). Shinke (1937) and Coleman (1940, 1941) have shown that such heteropycnotic regions are parts of the chromonema which remain tightly coiled or become precociously coiled. This could be confirmed in the present paper for the "knobs" of the meiotic chromosomes of Orphulella. Thus, one more "chromomere" was reduced to chromonematic coiling. There remained the "ultimate chromomere" of Belling (1928), the only bona fide "chromomere" according to most modern cytologists. This "chromomere" can only be seen in meiotic prophase and in salivary chromosomes of dipteran larvae, where the chromonemata are assumed to be maximally stretched. Let us examine point for point the evidence which is given for the reality of these "chromomeres" (see reviews cited in introduction).

(*a*) *"The chromomeres are seen in living cells and cannot be artefacts."* Bělař (1928) described "chromomeres" in living spermatocytes of the grasshopper. An analysis of his figure shows that he did not see chromomeres but the coils of diakinesis chromosomes. Lewis and Robertson (1916) and Chambers (1924) found "chromomeres" in the leptotene of living grasshopper spermatocytes. This may show that the structures observed are not fixation artefacts, but it certainly is easier to misinterpret narrow coils as granules in unstained cells where the chromosomes are hardly visible, than in well stained preparations. Yet there is a very interesting observation by Chambers (1924, page 270) which seems to have been overlooked by himself as well as most reviewers of chromosome structure. He writes: "If one of the early prophase chromosomes with ragged granular outlines be seized with a needle and rapidly pulled across the field so as to stretch it, the granules disappear and the whole substance becomes homogeneous." So Chamber's microdissection study does not support the "chromomere" hypothesis, but rather the assumption of a uniform but coiled leptotene chromosome.

(*b*) *"The chromomeres have specific and constant sizes and form a definite pattern."* The classical examples are Dendrocoelum (Gelei, 1921) and Phrynotettix (Wenrich, 1916). The observed patterns in these and other forms are an expression of the longitudinal differentiation of the chromosome. This differentiation is real. But the nature of this differentiation now turns out to be differential coiling and not a sequence of discrete bodies of different sizes. The large "chromomeres" in Phrynotettix are heterochromatic regions along the chromosome similar to those found in certain plant chromosomes and those described for Orphulella in this paper. In *Veltheimia viridifolia* Coleman (1940) could show that such heterochromatic regions are closely coiled sections of the chromonema. They correspond in structure to the differential segment in Rhoeo (Coleman, 1941) and the chromocenters in various animals and plants (Shinke, 1937). The knobs in maize are most probably of a similar nature.

(*c*) *"The chromomeres of homologous chromosomes pair specifically at zygotene."* Just as the turns in the spiral give the impression of "chromomeres" at leptotene, the paranemic spiral of the paired bivalent simulates a row of paired granules. Since homologous regions of the chromosomes pair, it is evident that heterochromatic sections will come to lie side by side in the pachytene chromosomes.

(*d*) *"The number of chromomeres in leptotene corresponds approximately to the number of genes in Lilium* (Belling, 1928). *In salivary chromosomes the*

bands, which correspond to the leptotene chromomeres, were shown to be closely associated with certain genes (Muller and Prokofyeva, 1935)."

Belling's estimate of the number of genes in Lilium was entirely arbitrary and he had no direct evidence for a correlation of "ultimate chromomeres" and genes. In salivary chromosomes of Drosophila, however, a great number of workers have proven beyond doubt that the visible "bands" are correlated with certain genes. A recent analysis of the salivary chromosomes of Sciara in collaboration with Dr. Helen Crouse (in press) has shown that the "granules" and "bands" are misinterpretations of a very complicated spiralization of a bundle of chromonemata. What has been described as a "chromomere" corresponding to a gene represents in reality a region of relatively considerable length along the chromonema. The cytogenetic work on Drosophila salivary chromosomes is not evidence for a "chromomeric" structure of the chromonema, but shows that certain sections of the uniform chromonematic thread correspond to definite genes and that the detailed nature of the coiling in these interphase chromosomes is closely correlated with a genetic specificity on a submicroscopic level.

In summary this is the evidence against the existence of "chromomeres": (a) In living cells the microdissection experiment of Chambers (1924) shows that the leptotene chromosome can be stretched into a uniform thread. (b) In several plants such as Tradescantia (Kaufmann, 1931; Swanson, 1943), Allium and Aloe (Koshy, 1934, 1937), Hyacinthus (Naithani, 1937), and in the grasshopper the leptotene chromosome consists of a uniform, coiled thread, Feulgen-positive throughout its length. No evidence of interchromomeric fibrils can be found. The leptotene coils can be followed into the pachytene where they increase in width and decrease in number. This explains the observation of many authors (e.g., Belling, 1931) that the "chromomeres" increase in size and decrease in number during the course of prophase. (c) In the diplotene chromosomes of the grasshopper no "chromomeres" can be seen in the large loops of the chromatids. If specific "chromomeric" granules were present at leptotene they should be visible also in the chromonema of diplotene. (d) McClintock (1944) has shown in maize that at least one gene is located in the interchromomeric thread between the terminal knob and the first "chromomere" on chromosome nine. This disproves definitely the idea, at least for maize, that the genes are necessarily located in the "chromomeres" which are connected by non-genic fibrils.

Diplotene chromosomes and "lamp-brush chromosomes"

The coiling cycle in the grasshopper appears to be identical with that described by Swanson (1942, 1943) for Tradescantia. The leptotene coil develops into the major coil of diakinesis and metaphase through despiralization. There is no definite minor coil, but from late pachytene on, an irregular waviness appears on the loops of the chromatids, resembling an incipient helix. A minor coil was seen in spermatocytes of another orthopteran, Podisma, by Makino (1936). In Trillium (Huskins, 1941) there seems to be a similar waviness instead of a definite helix as was demonstrated for Tradescantia. This difference in the appearance of the minor coil seems to be mainly one of timing of the spiralization cycle as Kuwada (1938) has suggested. In the grasshopper the chromatids have never been seen completely separated in diakinesis or metaphase. Their coils sometimes appear

interlocked as Kuwada (1938) found in Tradescantia, but this could not be definitely determined. Swanson (1942b) has shown that the terminalization of chiasmata is correlated with the despiralization of the major coil in Tradescantia. The same process takes place in the grasshopper and it is most likely that here, too, terminalization of chiasmata is the consequence of despiralization of the major coil.

The diffuse appearance of orthopteran as well as most other animal chromosomes in diplotene has made their analysis rather difficult. The chromonema is generally of smaller diameter than in plant chromosomes and therefore the delicate loops of the major coils escaped observation. This diffuse structure is due to a lateral separation of the chromatids in contrast to the usual appression of the chromatids in plant chromosomes. Under certain conditions, and especially in diakinesis, plant chromosomes also show a separation of chromatids. They then give the same pictures as diplotene chromosomes of animals (see the anaphase chromosome of desynaptic Trillium in Figure 9 of Sparrow, Huskins and Wilson, 1941; Swanson, 1942a, 1943, and Kuwada and Nakamura, 1938 for Tradescantia). Plant and animal chromosomes have often been described as reticulate or vacuolated. Grégoire and his school based on this their "vacuolization hypothesis" of chromosome structure. All their pictures can today be explained on the simple assumption of a multiple stranded helix with the chromonemata more or less appressed or opened up.

When the lateral separation of the chromonemata is great and the loops only faintly stained, the chromosome may appear covered with a layer of achromatic material (often described as "matrix" or "sheath"; see for instance Lee, 1921 and McClung, 1941, Figure 7). Probably many a "matrix" in the literature is nothing but the apparant connection between faintly staining outer loops, running at an even distance from the darker core of the chromosome where the chromonemata overlap. Makino (1936) published some photographs of diakinesis and metaphase chromosomes of Podisma which at first seem to contradict my interpretation of these stages. He shows a dark inner coil sometimes appearing double, surrounded by a light "matrix." Faint strands are sometimes seen to connect the central spiral with the border of the "matrix." Yet it is very easy to understand these figures with the help of a model of four wires coiled together. When two are maximally separated laterally and two stay appressed in the center, Makino's coil and matrix become explainable. The outer coils are not at all or only faintly stained in his gentian violet preparations and their outer boundary suggests the presence of a "matrix."

The previous studies of diplotene chromosomes of Orthoptera have completely ignored these outer gyres of the chromonemata. They were described as woolly threads or brushlike projections on the surface of the chromosome, but not as an essential part of it. Thus the pictures of Hearne and Huskins (1934), Nebel and Ruttle (1937), Darlington (1936), and the McClung school are based on optical illusions or too light staining. What were described as "chromomeres" in this stage are the points of overlap of the chromonemata. Darlington (1936) has studied relational coiling of chromatids and chromosomes in pachytene and diplotene. What he pictured as one single chromatid, however, is not a continuous structure, but a series of nodes of separate overlapping major coils. His relational coil of chromatids is therefore an optical illusion. Only a complete stretching of

the major coil could reveal whether the chromatids are wound around each other (see Kuwada, 1938).

Many oöcytes and spermatocytes in diplotene undergo a so-called "diffuse stage," which is correlated with the growth of the cell. The chromosomes stain only faintly and lose their definite shapes; they may even disappear into a reticular structure. In the grasshopper the diffuse nature of the chromosomes is due to the loosening and separation of the individual chromonemata of the major coil. This more or less pronounced loosening up of the gyres, combined possibly with some chemical changes in the composition of the chromatin, can explain the appearance of diplotene chromosomes during this stage in spermatocytes and oöcytes.

The diplotene chromosomes in the large oöcytes of some vertebrates have particularly interested the cytologist ever since their discovery by Rückert in 1892, because of their tremendous size. Their fuzzy and brush-like appearance warranted the name "lamp-brush chromosomes." Duryee (1937, 1938, 1939, 1941) has recently studied these chromosomes in great detail in the frog and salamander, and concludes that (1) they represent paired gelatinous cylinders in which the chromomeres are embedded. (2) From these chromomeres lateral loops grow out. He likens this growth to that of a crystal or the reproduction of a virus. (3) In a later stage, before the maturation divisions take place, these lateral loops are thrown off into the cytoplasm as genic products essential for the early embryo.

Painter (1940) came to somewhat different conclusions. He considers "lamp-brush chromosomes" to be chromosome aggregates, which originated through endomitosis and the loops to correspond to whole chromosomes. Material from thousands of such chromosomes, he maintains, is thrown into the cytoplasm as substrate for the synthesis of cleavage chromosomes. Koltzoff (1938) thinks that the lateral projections are side branches of the chromomeres which then are given off into the cytoplasm.

In contrast to Duryee, Koltzoff, and Painter, it is here suggested that "lamp-brush chromosomes" are typical diplotene chromosomes which differ from other diplotene chromosomes only in the tremendous longitudinal growth of the chromonemata. The loops are then the major coils of the laterally separated chromonemata, the "chromomeres" are simply overlaps of the strands just as in diplotene chromosomes of the grasshopper. Figure 12 shows a fragment of a "lamp-brush chromosome" of a frog oöcyte, smeared in aceto-orcein. The somewhat distorted large loops of the major coil and the minor coil are easily visible.

The evidence for this interpretation may be summarized as follows: (a) The loops are continuous as Rückert (1892) has already observed. He followed the chromonema for several turns. He also pointed out that the granules ("chromomeres") are not real, but optical sections of the overlapping threads. The denser inner region of the chromosome he described as due to the radial arrangement of the threads. (b) "Lamp-brush chromosomes" are diplotene chromosomes and except for their greater size have the same appearance as the diplotene chromosome of the grasshopper. Since it has been shown here that the loops are simply the gyres of the major coil of the separate chromonemata, one can conclude that the corresponding appearance of the "lamp-brush chromosome" is the result of a similar structure. (c) Koltzoff (1938) has published drawings of cross sections of "lamp-brush chromosomes" (his Figure 10, b and c). These cross sections look like a star with characteristically eight rays. These eight rays are most likely the eight

half-chromatids which form independent loops, though Koltzoff saw them as brush-like projections.

The reduction in chromosome size just before the meiotic divisions is accomplished then not by throwing off parts of the chromosome or entire chromosomes, but by elimination of material on a submicroscopic level.

The microscopic organization of chromosomes

Kuwada (1939) in his review of chromosome structure predicted that the spiral theory might well prove capable of harmonizing the various hypotheses of chromosome structure. Such a uniform interpretation of the structure of all types of chromosomes is now possible. The unit of the chromosome is the chromonema, a microscopically uniform thread. This chromonema is never completely straightened out, but always shows some degree of spiralization. This coiling is not at random, but, as the salivary chromosomes and heterochromatic regions show, is an expression of the longitudinal differentiation of the chromonema and closely correlated with the genes. It is, in other words, an expression of submicroscopic structure and possibly the functional state of the gene (cf. heterochromatin). The microscopic uniformity of course does not exclude a great variability of submicroscopic structure and chemical composition along the chromonema. During the mitotic cycle there develops a condensed chromosome through despiralization of the incipient coil of early prophase. The differentiation of mitotic chromosomes, primary and secondary constrictions, satellites, and heterochromatic regions are expressions of the differential coiling of the chromonemata. In the resting nucleus of different tissues we often find different patterns of heterochromatin. It may be that differential spiralization of the chromonemata in resting cells is correlated with cell differentiation. The chromonema is not uniform in length, but it can vary greatly from cell to cell in the same organism, as well as in the same cell in different metabolic states. In many synthetically very active cells as for instance some oöcytes, nurse cells, gland cells (dipteran salivary glands), the total amount of chromatin is greatly increased. This is accomplished by an increase in the number of chromosomes (endomitosis, cf. Geitler, 1941), by a growth in length of the chromonemata (as in "lamp-brush chromosomes") or by both simultaneously (salivary chromosomes). In "lamp-brush" and salivary chromosomes the increase in length is tremendous and would be difficult to understand if only inert "genoplasm" or "matrix" (Koltzoff, 1938) had increased. More likely it is an increase in the volume of the gene complex, related to the greater metabolic activity. We have to look at the gene, therefore, not as a unit of constant and specific size as expressed in the "chromomere" hypothesis, but as a complex that is greatly variable in mass, depending on the metabolic activity of the nucleus.

Summary

1. "Chromomeres" do not exist as definite structures. What has been described as "chromomeres" are (a) misinterpretations of gyres of the chromonematic helix (leptotene, somatic prophase); (b) points of overlap of chromonemata (diplotene); (c) heterochromatic sections consisting of more tightly coiled regions of the chromonema. The fundamental unit of the chromosome is a microscopically

uniform thread. The longitudinal differentiation of the chromosome is due to differential coiling of this chromonema.

2. "Lamp-brush chromosomes" are typical diplotene chromosomes, but with tremendously enlongated chromonemata. The side branches are the gyres of the major coils of the individual chromonemata, which have laterally separated from each other.

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